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# Pre-natal stress amplifies the immediate behavioural responses to acute pain in piglets

Kenneth M. D. Rutherford<sup>1,\*</sup>, Sheena K. Robson<sup>1</sup>, Ramona D. Donald<sup>1</sup>, Susan Jarvis<sup>1</sup>, Dale A. Sandercock<sup>2</sup>, E. Marian Scott<sup>3</sup>, Andrea M. Nolan<sup>2</sup> and Alistair B. Lawrence<sup>1</sup>

<sup>1</sup>Animal Behaviour and Welfare, Sustainable Livestock Systems, SAC, West Mains Road, Edinburgh EH9 3JG, UK

<sup>2</sup>Division of Cell Sciences, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Glasgow G61 1QH, UK

<sup>3</sup>Department of Statistics, University of Glasgow, Glasgow G12 8QQ, UK  
\*Author for correspondence (kenny.rutherford@sac.ac.uk).

**Pre-natal stress (PNS) or undernutrition can have numerous effects on an individual's biology throughout their lifetime. Some of these effects may be adaptive by allowing individuals to tailor their phenotype to environmental conditions. Here we investigated, in the domestic pig *Sus scrofa*, whether one possible consequence of a predicted adverse environment could be altered pain perception. The behavioural response of piglets to the surgical amputation ('docking') of their tail or a sham procedure was measured for 1 min in piglets born to mothers who either experienced mid-gestation social stress or were left undisturbed throughout pregnancy. A behavioural pain score was found to predict the docked status of piglets with high discriminant accuracy. Piglets exposed to PNS had a significantly higher pain score than controls, and for each litter of tail-docked piglets, the average pain score was correlated with mid-gestation maternal cortisol levels. The data presented here provide evidence that the experience of stress *in utero* can result in a heightened acute response to injury in early life. Speculatively, this may represent an adaptive alteration occurring as a consequence of a pre-natal 'early warning' of environmental adversity.**

**Keywords:** piglet; pre-natal stress; pain; predictive adaptive response

## 1. INTRODUCTION

Extensive experimental and epidemiological literature, in many species including humans, highlights the numerous effects that maternal stress during pregnancy can have on progeny phenotype. Some of these effects have been interpreted as having an adaptive basis, allowing offspring to adjust their phenotype to likely prevailing environmental conditions (i.e. to forecast the future; Bateson 2008). Such changes have been labelled 'predictive adaptive responses' and

differ from other forms of phenotypic plasticity, in that their fitness benefits occur at a later stage of development than that at which the actual change is provoked (Gluckman & Hanson 2004). Putative examples of such effects have been suggested in metabolic functioning and could also apply to early life alteration of stress reactivity. One area that has received only limited attention is the question of whether maternal stress could cause alterations in offspring sensitivity and responsiveness to pain. There is some evidence in rodent models that pre-natal stress (PNS) may alter the pain experience of juvenile or adult animals; for instance, through effects on offspring stress-induced analgesia or basal nociceptive thresholds (Szuran *et al.* 1991; Sternberg & Ridgway 2003). Pre-natal stress also increased the severity of the spontaneous behavioural response to formalin injection—a standard laboratory pain assay—in rats (Butkevich & Vershinina 2003).

Tail-docking of piglets is carried out on many pig farms in an effort to prevent the abnormal behaviour of tail-biting and involves the surgical removal of a large proportion of the piglet's tail without anaesthesia or analgesia in the first few days of life. Behavioural and physiological responses immediately following tail-docking suggest that piglets experience some degree of acute distress (Noonan *et al.* 1994; Sutherland *et al.* 2008). The frequency of tail-wagging, tail 'jamming' (where the remaining stump is clamped tightly between the hind limbs) and grunting vocalizations were all significantly increased in piglets following docking (Noonan *et al.* 1994), albeit relatively transiently. In another study (Sutherland *et al.* 2008), docked piglets were found to spend more time either sitting or dragging their rear along the ground while in a sitting posture during the hour after docking and also showed elevated cortisol levels for 60 min after the procedure compared with piglets that underwent a sham procedure.

This experiment aimed to determine whether a PNS treatment that had previously been shown to affect offspring growth, behaviour and stress reactivity (Jarvis *et al.* 2006) also impacted on the immediate behavioural responses of piglets to tail-docking.

## 2. MATERIAL AND METHODS

Thirty-six primiparous sows were kept in groups of six and artificially inseminated with monthly intervals between successive groups. Three groups of six were allocated to a stress treatment, with the remaining three groups acting as controls. Sow age and weight at insemination were balanced across treatment groups, and monthly inseminations alternated between stress and control groups. During pregnancy, the stress treatment sows were exposed to social stress, where each group of six was split into subgroups of three, each of which was mixed with three older multiparous sows, on two different occasions (approx. days 39–45 and 59–65 of the 114 day gestation; sows did not have their oestrus cycle artificially synchronized, so mix dates for individuals varied but all mixes took place in the second third of pregnancy). This commercially relevant procedure causes a profound social defeat (Jarvis *et al.* 2006) and, as a consequence, is highly stressful. For the period between the two mixes, the two subgroups were reunited. To assess the severity of the mixes, measurements of body weight, body lesions, home pen behaviour and salivary cortisol were taken from stressed and control sows (electronic supplementary material). Nine sows did not become pregnant at the appropriate time so the final sample size was 27 (16 stressed and 11 control). Five days before parturition, sows were moved to standard farrowing crates. All piglets were weighed on the first day of life, and crown-to-rump length was also measured to allow calculation of the ponderal index (a measure of weight to size proportionality). At around 3 days

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Table 1. Summary statistics (mean  $\pm$  s.e.) for mixed or control sows and their litters.

variable	mixed	control	statistics
<i>sows</i>			
weight gain (kg) over mix period	$-0.53 \pm 2.46$	$13.95 \pm 2.27$	$W = 10.31, p = 0.031$
body lesions at end of mix period	$83.6 \pm 9.6$	$14.2 \pm 2.8$	$W = 22.22, p = 0.007$
behaviour (% of daytime in bedded area) during mix period	$15.3 \pm 3.7$	$76.4 \pm 3.5$	$W = 32.69, p = 0.004$
salivary cortisol (ng ml <sup>-1</sup> )			
mix 1, day 1	$5.21 \pm 0.52$	$2.76 \pm 0.36$	$W = 12.48, p = 0.002$
mix 2, day 1	$4.99 \pm 0.53$	$2.32 \pm 0.20$	$W = 15.99, p < 0.001$
<i>piglets</i>			
litter size	$12.1 \pm 0.81$	$11.5 \pm 0.98$	$W = 0.28, p = 0.62$
day 1 weight (kg)	$1.45 \pm 0.04$	$1.49 \pm 0.07$	$W = 0.47, p = 0.53$
day 1 ponderal index	$72.9 \pm 2.07$	$72.5 \pm 2.96$	$W = 0.01, p = 0.92$

of age, all piglets were tail-docked (stressed:  $n = 94$ , control:  $n = 51$ ) or sham-docked (stressed:  $n = 75$ , control:  $n = 51$ ). For docking or the sham procedure, individual piglets were placed in a plastic box (base: 48 cm  $\times$  64 cm). For docking, half of the tail was removed using a clean pair of surgical cutters. Following docking or the sham handling procedure, the piglet was left undisturbed in the box for 1 min and its behaviour was recorded using a digital video camera. The 1 min video clips of piglet behaviour were later scored using OBSERVER analysis software (Noldus), according to a detailed ethogram (electronic supplementary material). All video clips were scored by a single observer (one of the authors: S.K.R.) who was familiar with the experimental design but who was blind to the stress status of piglets during behaviour scoring. Discriminant analysis with cross-validation (MINITAB 15) was used to identify the combination of behaviours that best discriminated between the sham- and tail-docked control groups and which thus could be used to characterize the response to tail-docking. Residual maximum likelihood (GENSTAT 9) was used to compare scores between treatment groups. For litter traits, maternal treatment (stressed versus control) was fitted as a fixed effect and sow group was fitted as a random effect. For piglet behaviour, maternal treatment, sex, day 1 weight and ponderal index of each piglet were fitted as fixed effects with litter fitted as a random effect. Wald statistics and significance values have been calculated for each variable after all other variables had been accounted for. Pearson's correlation coefficients were calculated between mean sow salivary cortisol on different days of the mixes and the mean litter behavioural pain score for tail-docked litters.

### 3. RESULTS

The social mixes represented a profound stress treatment: mixed sows showed elevated salivary cortisol, reduced weight gain, increased body lesions and spent less time in the straw-bedded area of their pen over the period of the two mixes compared with undisturbed controls (table 1). There was no effect of maternal stress on litter size, or on piglet weight, or ponderal index (table 1).

Several behavioural parameters clearly altered as a result of docking. Cross-validated discriminant analysis (electronic supplementary material) showed that a combination of frequency of tail-wagging and the duration spent in particular tail states (clamped or rigid) correctly classified 88.2 per cent of control piglets as docked or sham-docked with misclassification rates of 11.8 and 0.0 per cent, respectively. The combination of tail-wagging frequency and duration of abnormal tail states was, therefore, used as a behavioural pain score to describe the severity of piglet docking pain.

Offspring from stressed mothers had a significantly higher pain score following tail-docking, compared

with offspring from control mothers (PNS =  $37.5 \pm 1.8$ , control =  $25.3 \pm 2.3$ ;  $W = 4.92$ ,  $p = 0.027$ ). There was no effect of piglet sex ( $W = 0.02$ ,  $p = 0.88$ ), body weight ( $W = 0.03$ ,  $p = 0.85$ ), or ponderal index ( $W = 0.15$ ,  $p = 0.70$ ) on pain score, and no significant interactions were found. Maternal cortisol and average pain score for each litter were not correlated before or after the two mix periods (pre-mix:  $r^2 = 0.01$ ,  $p = 0.74$ ; post-mix:  $r^2 = 0.03$ ,  $p = 0.57$ ), when cortisol levels did not differ between the treatment groups (pre-mix:  $W = 0.3$ ,  $p = 0.59$ ; post-mix:  $W = 0.07$ ,  $p = 0.80$ ). However, litter pain score was correlated with maternal cortisol levels on the first day of each of the two mixes (mix 1, day 1:  $r^2 = 0.29$ ,  $p = 0.048$ ; mix 2, day 1:  $r^2 = 0.54$ ,  $p = 0.003$ ).

### 4. DISCUSSION

Piglet behaviour is immediately altered as a consequence of tail-docking, and the results here strongly suggest that the magnitude of this acute response is increased in piglets born to mothers who experienced social stress during pregnancy. This is, to our knowledge, the first demonstration in a non-rodent species that PNS can alter responses to noxious challenge in neonatal animals and also that experience of a social stressor during pregnancy can alter putatively pain-related behaviours in offspring. That the average pain score for each litter positively correlated with maternal cortisol levels is suggestive of a causal relationship between the severity of the dam's social stress experience and the severity of her offspring's acute response to neonatal injury.

It has been suggested that many of the effects of maternal stress on offspring biology have an adaptive basis (Gluckman & Hanson 2004). In this view, maternal stress signals to the developing offspring that it will be born into an adverse environment and provides an opportunity for closer matching between the offspring phenotype and prevailing environmental conditions. From a theoretical perspective, a more reactive phenotype, although physiologically costly, may be more suited to a risky environment (the 'smoke-detector principle'; Nesse 2005). Similarly, error management theory (Haselton & Nettle 2006) predicts that evolution should favour strategies that

minimize the most costly mistakes in any given scenario, not the most common. In a situation where an animal is exposed to a stimulus that could lead to further tissue injury, the most costly mistake—in survival terms—is to under-react. The evolutionary benefits of the capacity to feel pain are: (i) to act as a warning of damage or potential damage; (ii) to stimulate withdrawal from and future avoidance of the stimulus; and (iii) to motivate protective behaviour that aids healing following injury (Bateson 1991). All of these functions are even more imperative in a dangerous environment. Pain can be viewed as a motivational state, and the negative valence of pain allows it to inhibit competing behaviours where necessary for survival. The downside is that the experience of pain during injuries such as tail-docking, where there is little functional benefit to pain, may be heightened.

The results presented here represent some preliminary evidence that the immediate responses to acute pain are increased as a consequence of maternal pregnancy stress. Pain perception is dynamic and labile and is open to manipulation by the social and physical environment throughout life. The extent to which pre-natally induced changes might persist throughout an individual's lifetime remains to be determined. However, in rats, PNS increased the severity of the spontaneous behavioural response to formalin through to adulthood (Butkevich & Vershinina 2003).

In conclusion, the severity of the acute behavioural response to tail-docking is increased in piglets born to mothers that were stressed during pregnancy, suggesting that these pre-natally stressed individuals may have a heightened experience of pain. Individual variation in the severity of pain experienced as a consequence of a standard noxious challenge can be generated by variation in the foetal environment, in this experiment brought about through different maternal experiences of social stress during pregnancy. Although the proximate mechanisms generating such an effect are not yet known, the ultimate function may be to create an offspring phenotype that is better prepared for survival in adverse environments.

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